Implementing the EC roadmap to phasing out animal testing for the assessment of endocrine-disrupting properties of pesticides for the environment: Challenges and opportunities

Laurent Lagadic[1]

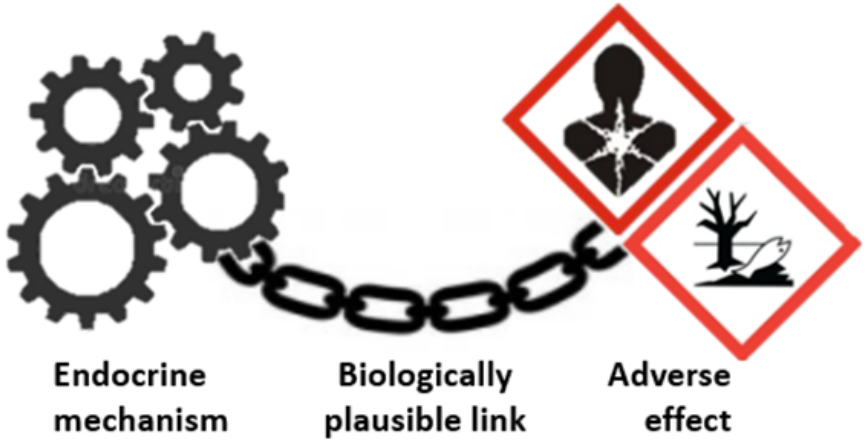
with contributions from Helen Tinwell[1], Lennart Weltje[2] and James R. Wheeler[3]

[1]Bayer AG Crop Science R&D, Germany
[2]BASF Agricultural Solutions Deutschland GmbH, Germany
[3]Corteva agriscience, The Netherlands

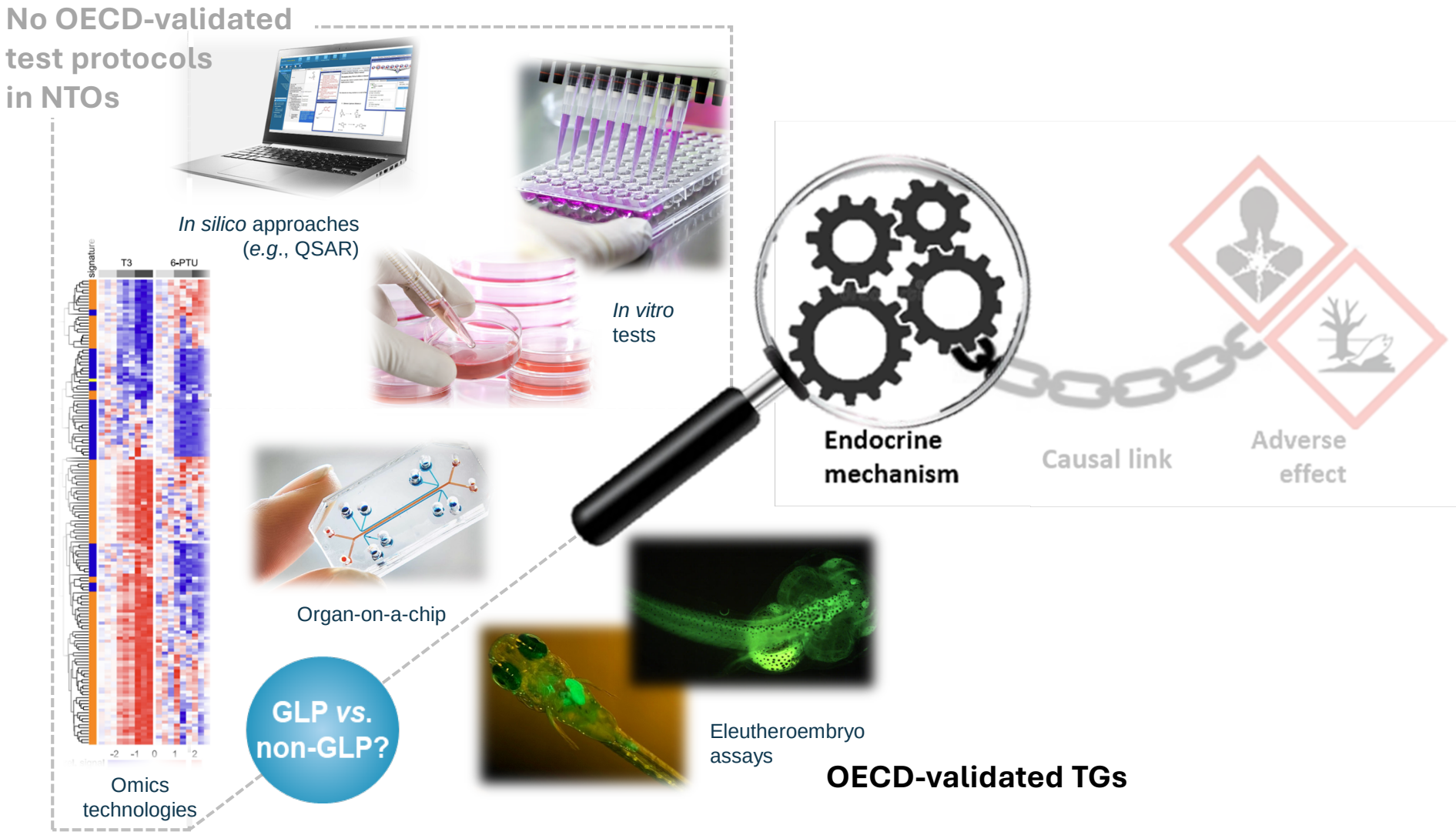


NAM toolbox for environmental endocrine disruptor (ED) assessment

Definition of an endocrine disruptor



NAM toolbox for environmental endocrine disruptor (ED) assessment



NAM toolbox for environmental endocrine disruptor (ED) assessment

No OECD-validated test protocols in NTOs

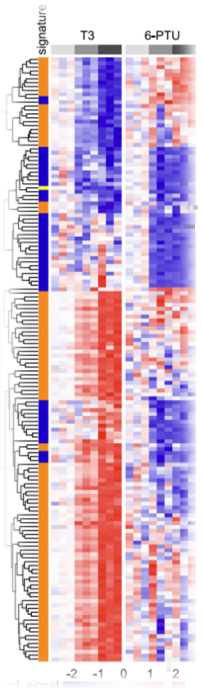


In silico approaches (*e.g.*, QSAR)

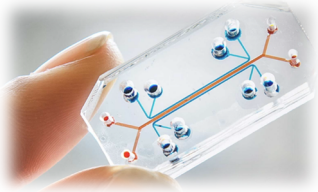




In vitro tests

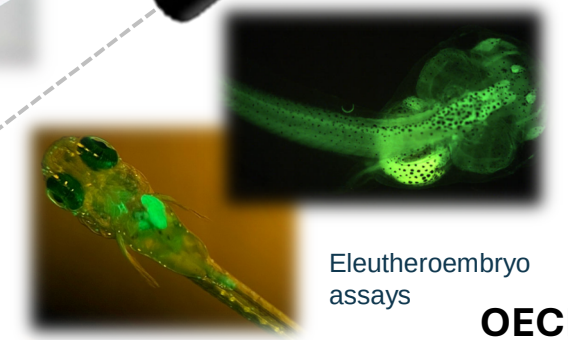


Omics technologies

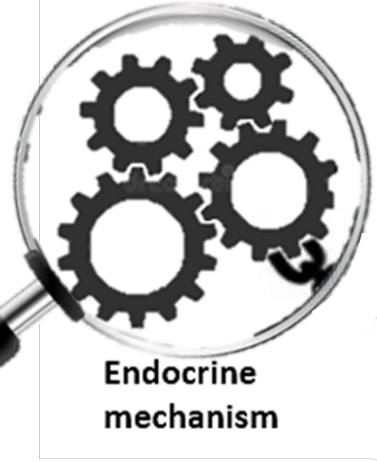


Organ-on-a-chip

GLP *vs.* non-GLP?



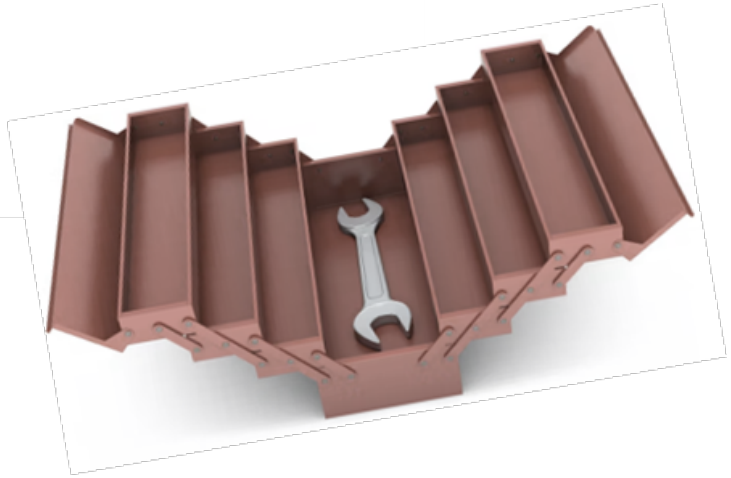
Eleutheroembryo assays



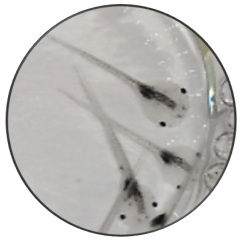
Endocrine mechanism

OECD-validated TGs

The NAM toolbox is currently very limited in ecotoxicology for non-mammalian species.

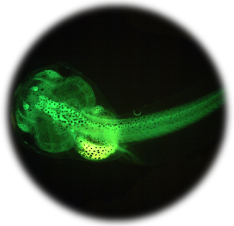


Available OECD test guidelines using transgenic eleutheroembryos



Xenopus laevis

- *Xenopus* Eleutheroembryonic Thyroid Assay (**XETA**); OECD TG 248 (June 2019)
- Annex A to the ECHA-EFSA ED Guidance published in April 2021 provides recommendations on when to use the XETA in thyroid disruption assessment strategies







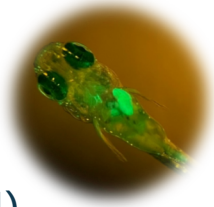
Zebrafish

- Detection of Endocrine Active Substances, acting through estrogen receptors, using transgenic tg(cyp19a1b:GFP) Zebrafish embrYos (**EASZY assay**); OECD TG 250 (June 2021)



Medaka

- Rapid Androgen Disruption Activity Reporter (**RADAR**) **assay** using medaka embryos; OECD TG 251 (June 2022)

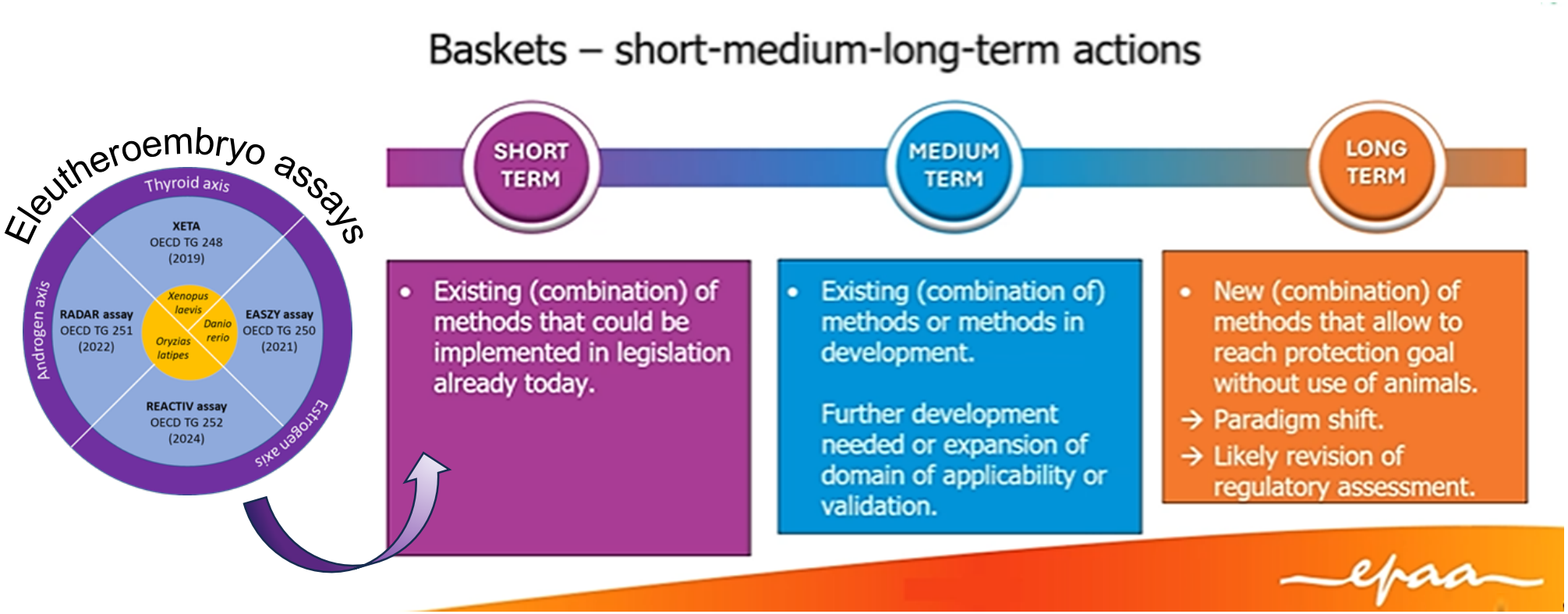


- Rapid Estrogen ACTivity *In Vivo* (**REACTIV**) **assay** also using medaka embryos; OECD TG 252 (June 2024)

No guidance on how/when to use fish eleutheroembryo assays in testing strategies for EAS modality

Pictures courtesy WatchFrog Laboratory, France

European Commission roadmap towards phasing out animal testing for chemical safety assessment



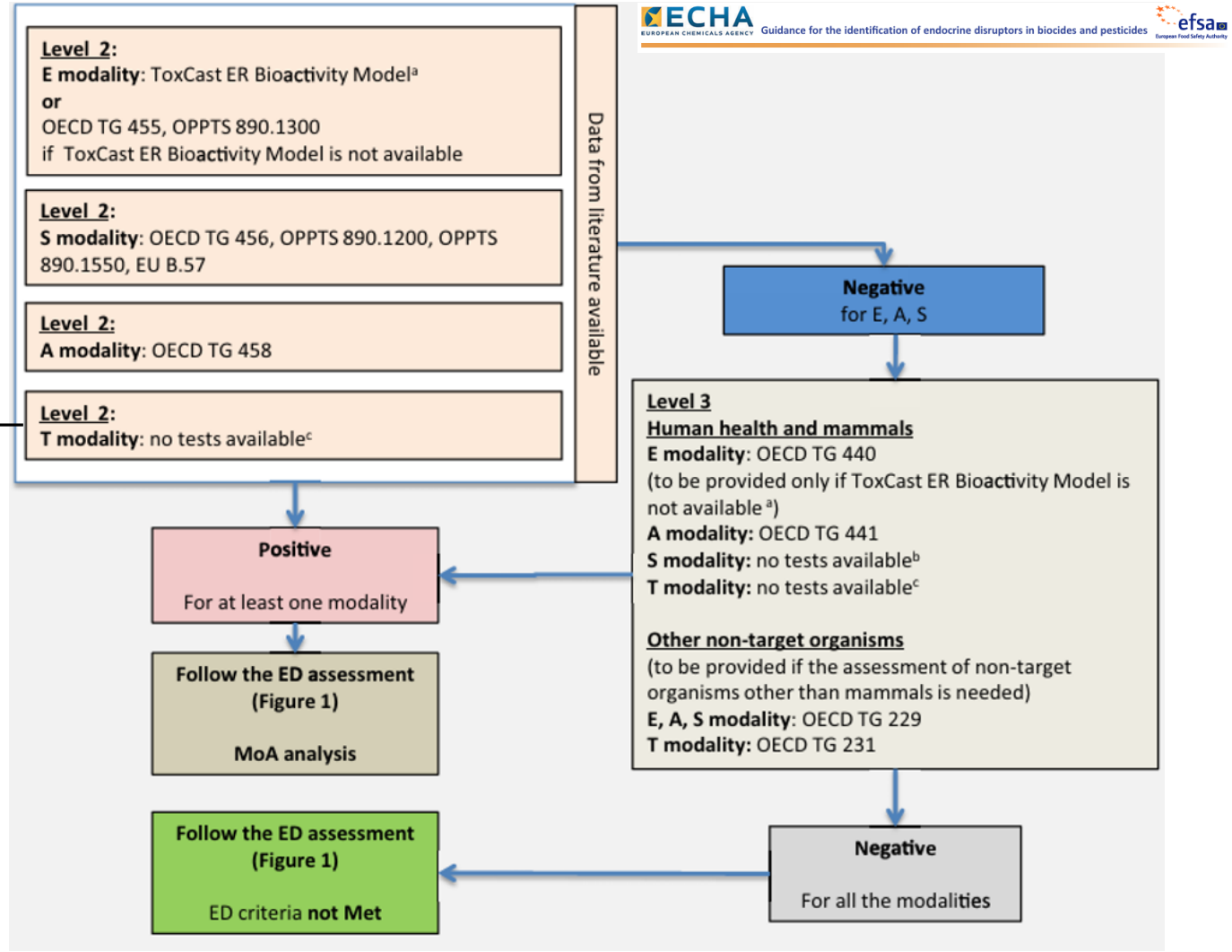
In the long-term, animal-free approaches not relying on embryos are preferred.

In vivo tests, including those based on eleutheroembryos, are considered as an intermediate solution and only if it reduces the overall need for animal testing until a full replacement is possible.

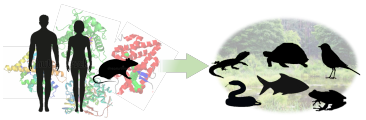
Current practice: use of mammalian *in vitro* data to evaluate endocrine activity in non-mammalian species

PUBLIC-PRIVATE PLATFORM FOR THE VALIDATION OF ENDOCRINE DISRUPTORS CHARACTERIZATION METHODS
Pepper

TPO	Pepper project
NIS	Pepper project to start autumn 2026
DIO 1	Pepper project; SPSF submitted to OECD
DIO 2	?
DIO 3	?
TTR	Pepper project; SPSF submitted to OECD
UGT-T4 activity	Submitted to Pepper



Challenge: overcome the current limitations in non-animal methods relevant for environmental ED assessment

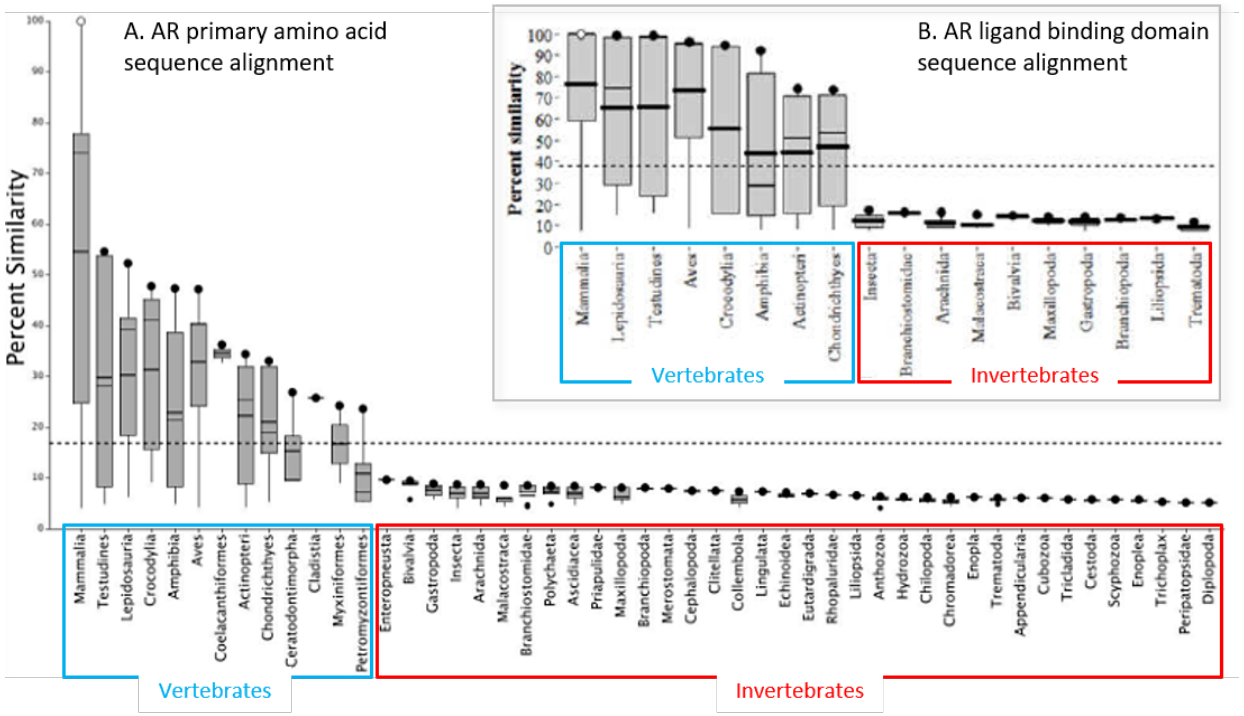


Cross-species extrapolation

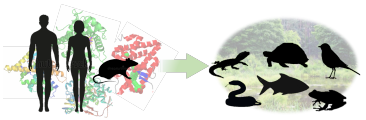
In principle, endocrine pathways are highly conserved across vertebrate taxa, so there is a good potential for read-across from mammals to non-mammalian taxa, and vice-versa.

Sequence **A**lignment to **P**redict **A**cross **S**pecies **S**usceptibility (**SeqAPASS**). Data showing the percent similarity across species compared to *Rattus norvegicus* for (A) the primary amino acid and (B) ligand binding domain sequence alignment of the androgen receptor (AR).

From LaLone *et al*. 2018. *Environ. Sci. Technol.* 52:13960-13971. www.seqapass.epa.gov/seqapass



Challenge: overcome the current limitations in non-animal methods relevant for environmental ED assessment



Cross-species extrapolation

- Extrapolations based only on existing knowledge and theoretical assumptions not (always) appropriate; reality check using empirical data is crucial.
  ⇒ read-across to be used wisely; potency matters!

- Define limitations of cross-species extrapolation
  ⇒ *biologically plausible* taxonomic domain of applicability from *in silico* (computational) tools (*e.g.*, SeqAPASS) may differ from *empirical* taxonomic domain of applicability.
  ⇒ ADME, TK/TD, protein binding may complicate extrapolation.

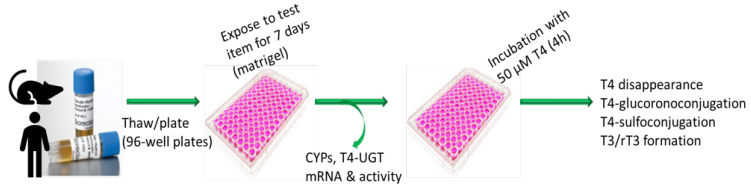
- Case-example: inducibility and role of thyroid hormone conjugating liver enzymes in amphibians
  ⇒ mammalian UDPGT inducers inactive in the XETA.
  ⇒ apparent insensitivity of the AMA to liver enzyme inducers.

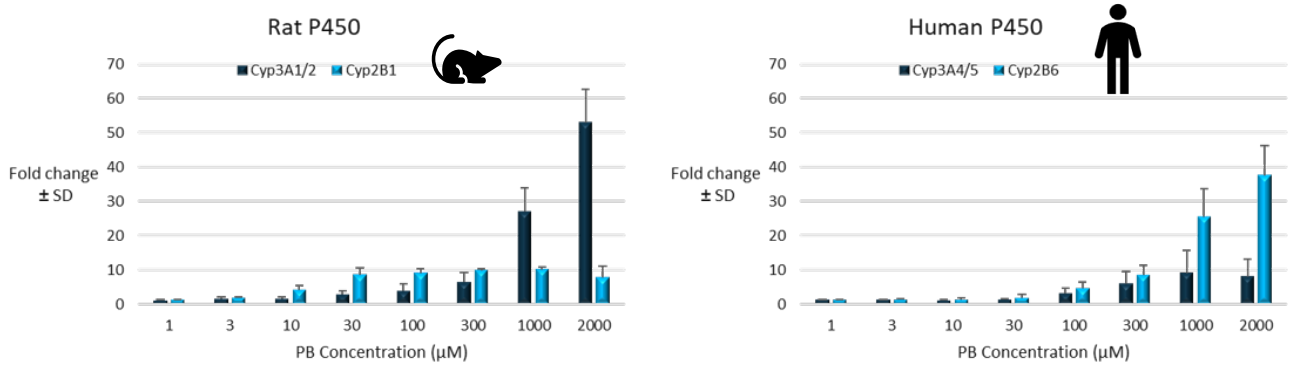


Challenge: overcome the current limitations in non-animal methods relevant for ED assessment

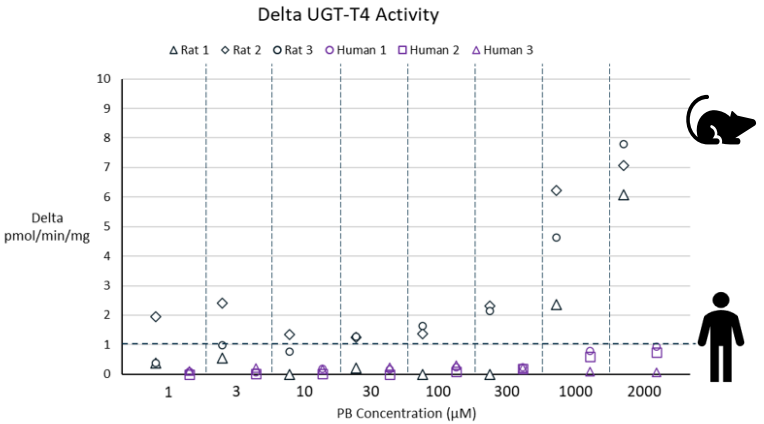
Cross-species extrapolation

// Rat to human extrapolation is not always straightforward.





Clear induction of Phase I (CYP450) hepatic enzymes in both species



Clear induction of UGT-T4 in rat but not human hepatocytes

⇒ Assay not yet validated but high data convergence.
Nevertheless, high degree of uncertainty, thereby preventing conclusions concerning human non-relevance being drawn.

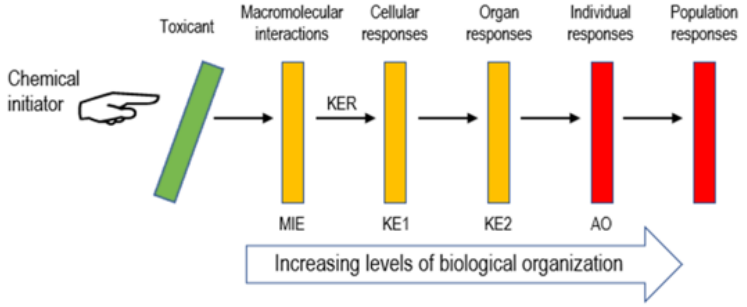
Challenge: overcome the current limitations in non-animal methods relevant for ED assessment

Adverse Outcome Pathways (AOPs)

- AOPs are not extrapolation tools; a Molecular Initiating Event (MIE) does not necessarily result in an Adverse Outcome; thresholds exist!
  ⇒ develop quantitative AOPs (qAOPs) for predictive purposes

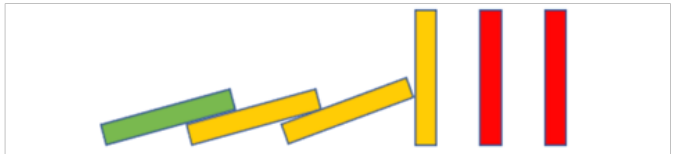
- Pre-defined AOPs available in AOP-Wiki cannot be assumed by default, from a MIE identified *in vitro*
  ⇒ confront AOP Key Events with empirical data

- Many ED-related AOPs, fewer non-ED-related AOPs in AOP-Wiki
  ⇒ develop non-ED-related AOPs to support postulated non-ED MoA



Typical representation of an AOP (*e.g.* US EPA Fact Sheets).

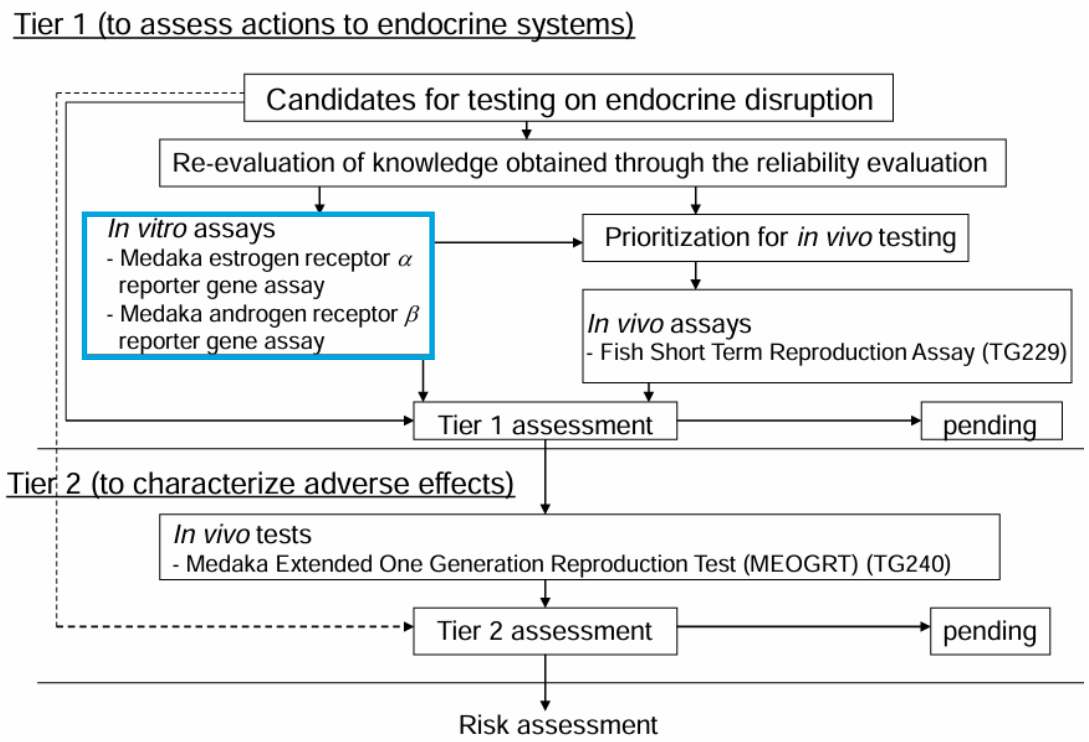

Interpretation of the domino effect that may result from the typical representation of an AOP.


Situation of interrupted cascading effect that can happen in real life.

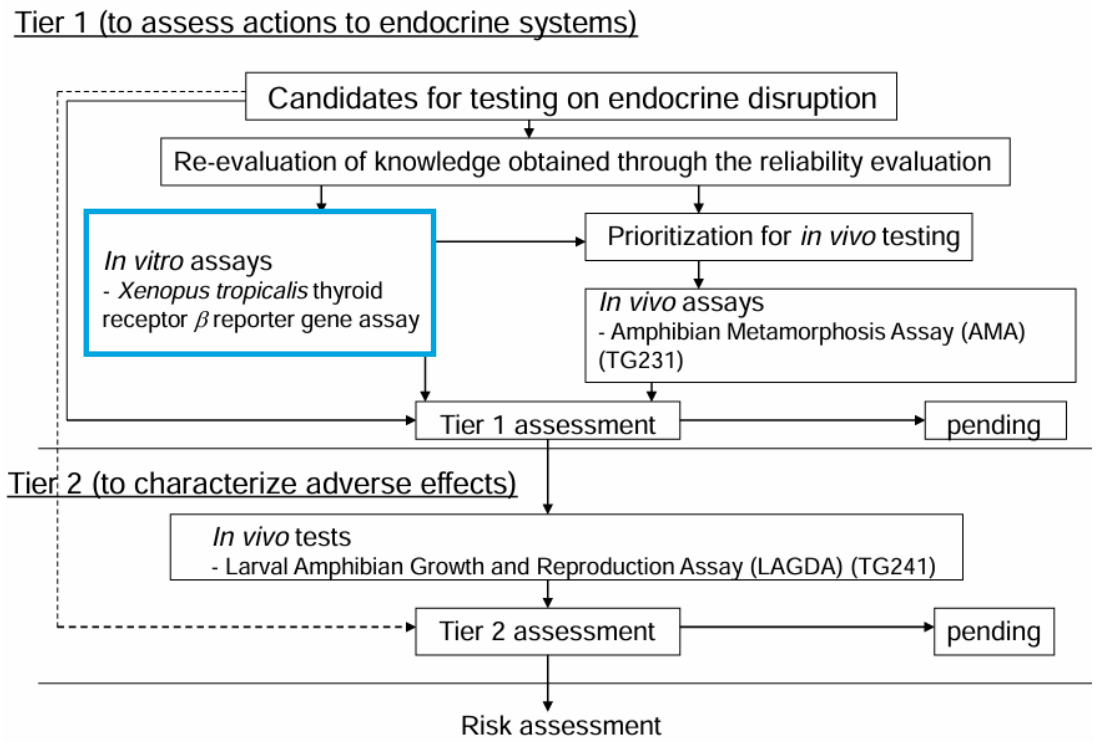
Opportunity: development and validation of *in silico* and *in vitro* methods for non-mammalian species

Japanese EXTEND programme

Estrogenic, anti-estrogenic and androgenic activities



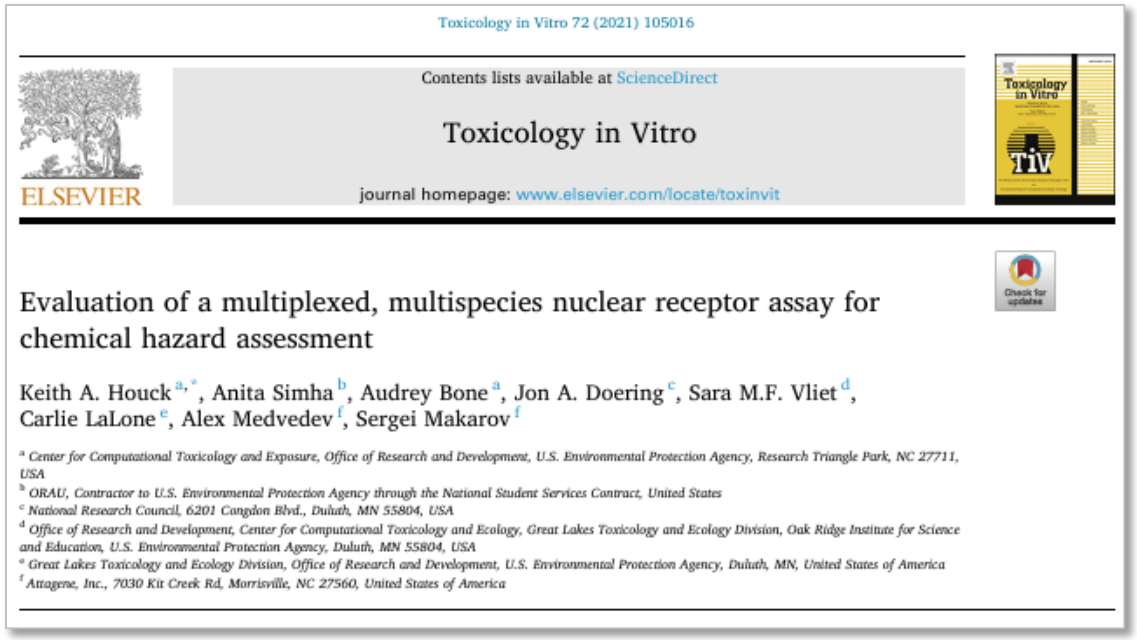
Thyroid & anti-thyroid activities



From K. Yamazaki, Chem Academy 7th International Conference on endocrine disruptors, 2022.

Opportunity: development and validation of *in silico* and *in vitro* methods for non-mammalian species

ECOTOX-FACTORIAL™



Toxicology in Vitro 72 (2021) 105016

Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

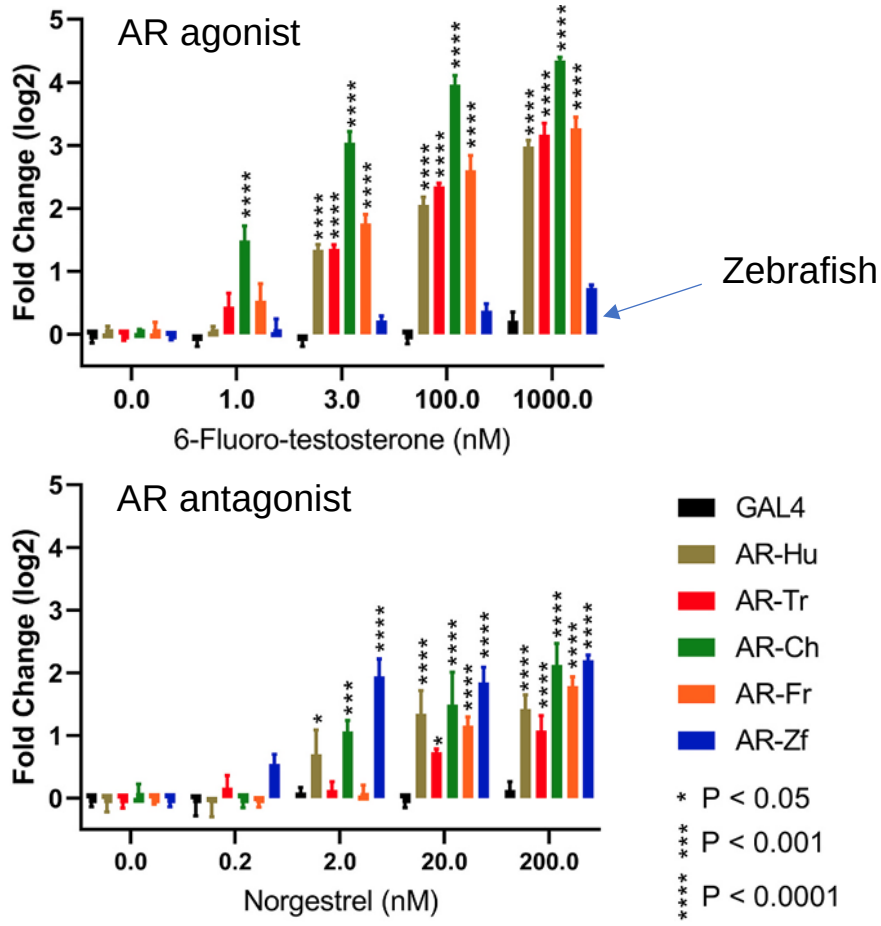
ELSEVIER

Evaluation of a multiplexed, multispecies nuclear receptor assay for chemical hazard assessment

Keith A. Houck [a,*], Anita Simha [b], Audrey Bone [a], Jon A. Doering [c], Sara M.F. Vliet [d], Carlie LaLone [e], Alex Medvedev [f], Sergei Makarov [f]

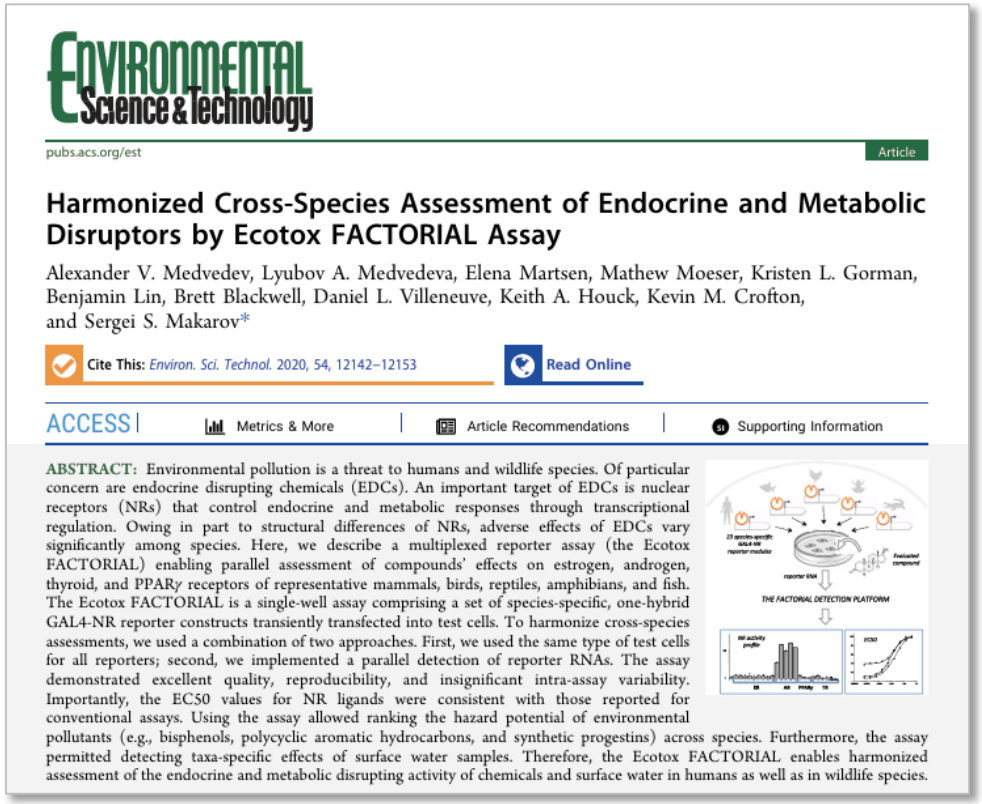
[a] Center for Computational Toxicology and Exposure, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711, USA
[b] ORAU, Contractor to U.S. Environmental Protection Agency through the National Student Services Contract, United States
[c] National Research Council, 6201 Congdon Blvd., Duluth, MN 55804, USA
[d] Office of Research and Development, Center for Computational Toxicology and Ecology, Great Lakes Toxicology and Ecology Division, Oak Ridge Institute for Science and Education, U.S. Environmental Protection Agency, Duluth, MN 55804, USA
[e] Great Lakes Toxicology and Ecology Division, Office of Research and Development, U.S. Environmental Protection Agency, Duluth, MN, United States of America
[f] Attagene, Inc., 7030 Kit Creek Rd, Morrisville, NC 27560, United States of America

The TRANS-FACTORIAL™ system of human nuclear receptors has been modified to include additional species: mouse (*Mus musculus*), frog (*Xenopus laevis*), zebrafish (*Danio rerio*), chicken (*Gallus gallus*), and turtle (*Chrysemys picta*), and evaluated with 191 chemicals.



Opportunity: development and validation of *in silico* and *in vitro* methods for non-mammalian species

ECOTOX-FACTORIAL™



Environmental Science & Technology

pubs.acs.org/est Article

Harmonized Cross-Species Assessment of Endocrine and Metabolic Disruptors by Ecotox FACTORIAL Assay

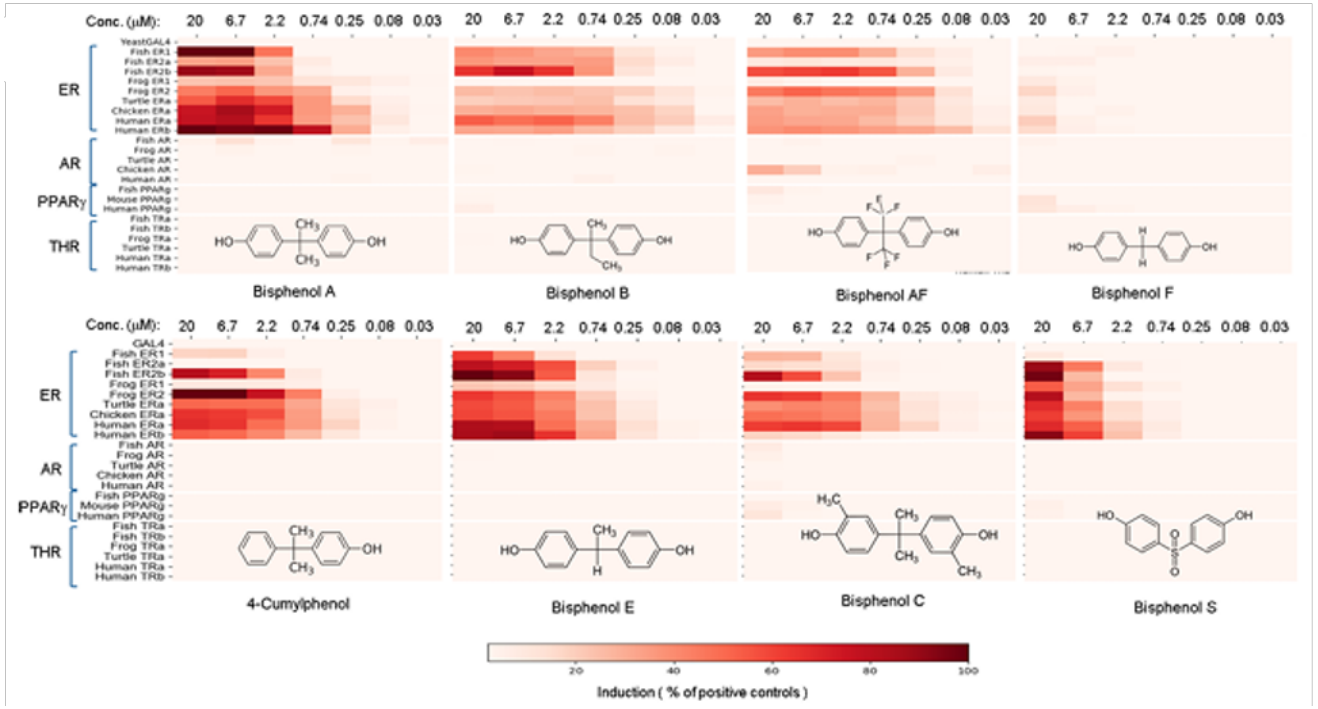
Alexander V. Medvedev, Lyubov A. Medvedeva, Elena Martsen, Mathew Moeser, Kristen L. Gorman, Benjamin Lin, Brett Blackwell, Daniel L. Villeneuve, Keith A. Houck, Kevin M. Crofton, and Sergei S. Makarov*

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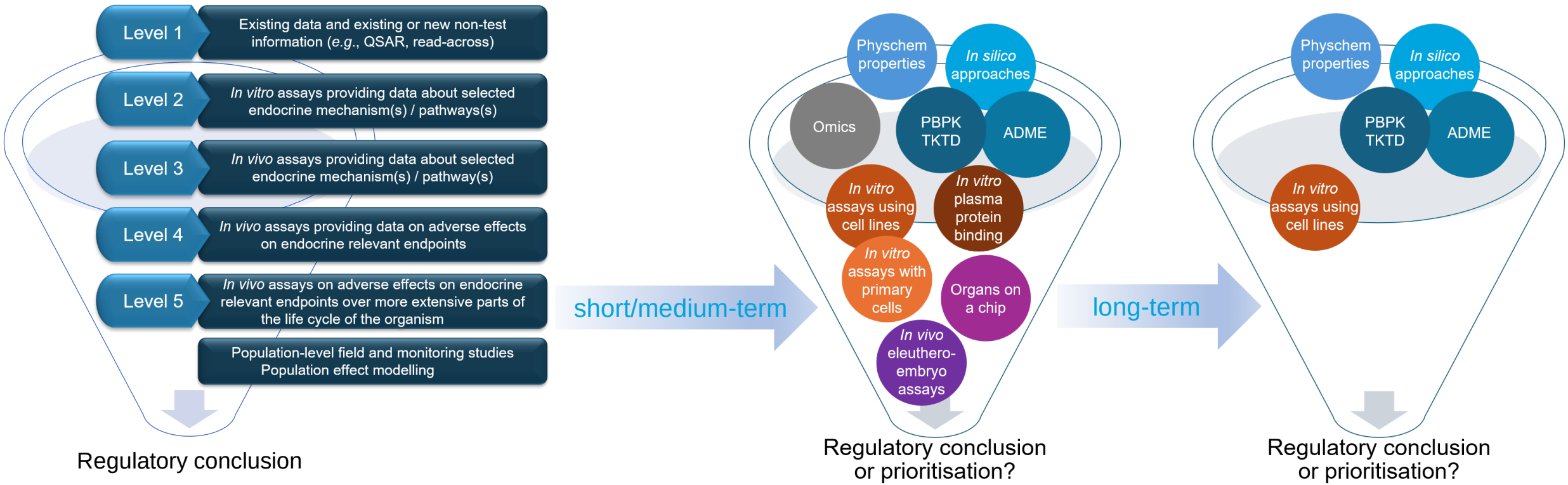
ABSTRACT: Environmental pollution is a threat to humans and wildlife species. Of particular concern are endocrine disrupting chemicals (EDCs). An important target of EDCs is nuclear receptors (NRs) that control endocrine and metabolic responses through transcriptional regulation. Owing in part to structural differences of NRs, adverse effects of EDCs vary significantly among species. Here, we describe a multiplexed reporter assay (the Ecotox FACTORIAL) enabling parallel assessment of compounds' effects on estrogen, androgen, thyroid, and PPARγ receptors of representative mammals, birds, reptiles, amphibians, and fish. The Ecotox FACTORIAL is a single-well assay comprising a set of species-specific, one-hybrid GAL4-NR reporter constructs transiently transfected into test cells. To harmonize cross-species assessments, we used a combination of two approaches. First, we used the same type of test cells for all reporters; second, we implemented a parallel detection of reporter RNAs. The assay demonstrated excellent quality, reproducibility, and insignificant intra-assay variability. Importantly, the EC50 values for NR ligands were consistent with those reported for conventional assays. Using the assay allowed ranking the hazard potential of environmental pollutants (e.g., bisphenols, polycyclic aromatic hydrocarbons, and synthetic progestins) across species. Furthermore, the assay permitted detecting taxa-specific effects of surface water samples. Therefore, the Ecotox FACTORIAL enables harmonized assessment of the endocrine and metabolic disrupting activity of chemicals and surface water in humans as well as in wildlife species.

Nuclear receptor response profiles across vertebrates for bisphenols.



Options for the future?

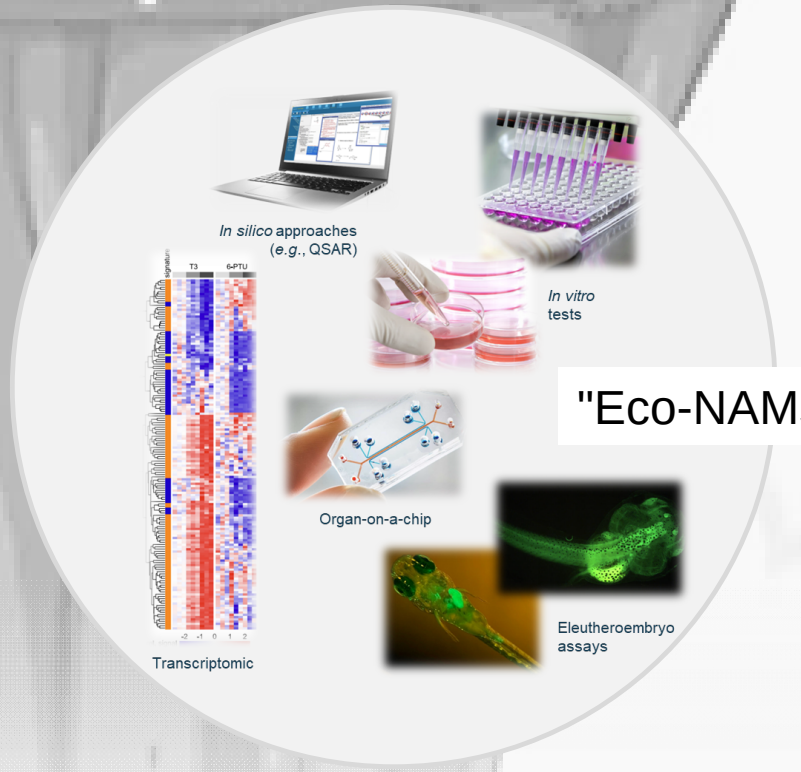
Current status: tiered or toolbox approach according to the OECD Conceptual Framework



// **How population-relevant adversity could be proven?**

Mind the gap!





"Eco-NAMs"

Population



Protection goal
and ultimate step of
environmental ED assessment

Thank you for your attention